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Cyanodermella asteris sp. nov. (*Ostropales*) from the inflorescence axis of *Aster tataricus*

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Abstract—An endophytic fungus isolated from the inflorescence axis of *Aster tataricus* is proposed as a new species. Phylogenetic analyses based on sequences from the ribosomal DNA cluster (the ITS1+5.8S+ITS2, 18S, and 28S regions) and the RPB2 gene revealed a relationship between the unknown fungus and the *Stictidaceae* lineage of the *Ostropales*. The new species, *Cyanodermella asteris*, grows in standard fungal growth media as a fluffy, pink filamentous fungus. Asexual and sexual sporulation has not yet been observed on media or in the plant.

Key words—*Ascomycota, Asteraceae, Lecanoromycetes, Pezizomycotina*

Introduction

Aster tataricus (*Asteraceae*) is native to northern Asia in Siberia, Mongolia, Japan, Korea, and China. Its roots are well known in traditional Chinese and Japanese medicine due to their diuretic, antibacterial, antiviral, and anti-ulcer activities (Shao et al. 1997a, b; Shirota et al. 1997). Diverse secondary metabolites have been identified in *A. tataricus* roots: shionone-type triterpenes [e.g., astataricusones and astataricusol (Zhou et al. 2013); astershionones (Zhou et al. 2014)], cyclopeptides[e.g., astins (Morita et al. 1996, Xu et al. 2013)], and flavonoids [e.g., quercetin and kaempferol (Wang et al. 2003)].

With over 2700 species in eleven families, the *Ostropales* (Hibbett et al. 2007) represent a large part of the *Lecanoromycetes* (*Ascomycota*, *Pezizomycotina*). Although most ostropalean fungi are lichenized, parasites and saprotrophs are also known (Grube & Hawksworth 2007; Lutzoni et al. 2004; Sherwood 1977a, 1977b; Wedin et al. 2004, 2006). Baloch et al. (2010), who outline the current taxonomy of the *Ostropales*, note that most *Stictidaceae* are saprotrophs and form very small fruiting bodies.

Here we describe our isolation of an endophytic fungus from the inflorescence of *Aster tataricus* and demonstrate its placement in the *Ostropales*. DNA sequences from the newly discovered fungus matched no previously sequenced species, although it did show phylogenetic affinities with *Cyanodermella*. For that reason, we propose this endophyte as a new species, named here *Cyanodermella asteris*.

Materials & methods

Chemicals were purchased from Duchefa (Haarlem, The Netherlands) and Roth (Karlsruhe, Germany). Plant hormones were obtained from Duchefa and Sigma-Aldrich (Hamburg, Germany) and PCR reagents from Thermo Fisher Scientific (Waltham, USA).

Collecting & field sites

The *Aster tataricus* host plant was obtained from Sarastro Stauden (Ort im Innkreis, Austria) and cultivated in the Plant Physiology laboratory greenhouse, Technische Universität Dresden, Germany. The cultivar is henceforth referred to as *A. tataricus* cv. Austria.

Isolation

The fungus was isolated from surface-sterilized inflorescence axes of *Aster tataricus* cv. Austria. After the axes were treated first for 30 s with 70% ethanol + 0.1% Triton X-100, then for 5–7 min with 4.2% sodium hypochlorite + 0.1% Triton X-100, and washed 3 times with autoclaved distilled water, samples were cultivated

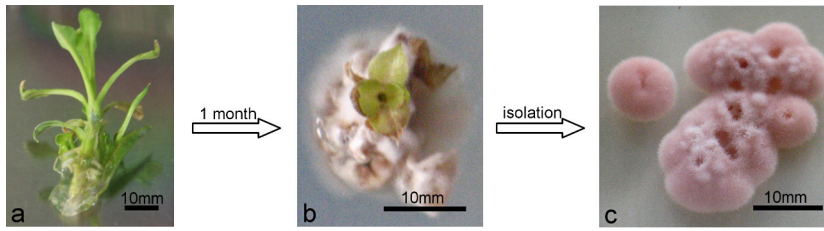

Fig. 1. Isolation of the new endophyte *Cyanodermella asteris*. a. During the first few weeks, the new endophyte *C. asteris* was isolated from sterile plantlets growing on hormone containing MS medium. b. Antimycotic treatment did not reduce the fungal outgrowth, and the fungus still appeared on the plant surface and grew on the MS medium. c. Isolation and cultivation on potato dextrose agar produced a fluffy pink filamentous fungus.

on Murashige-Skoog (MS) medium (Murashige-Skoog 1962; 4.4 g/l MS medium including vitamins, 3% sucrose, 1% phyto agar, pH 5.8) including auxins and/or cytokinins (i.e., naphtylacetic acid, indole-3-butyric acid, 6-benzylaminopurine) usually used to stimulate sterile plants in vitro. The MS plates were incubated in long day cycles (16 h light, 23°C; 8 h dark, 18°C). After several transfers of sterile plantlets to fresh MS plates, we found a fungus growing from the small plants into the MS medium. Initially considered a contaminant, the fungus was treated with antimycotics (50 µg/ml Nystatin® for 4 months and penicillin/ streptomycin/ amphotericin B solution for 1 month). However, the treatment did not eliminate the fungus from the plant culture. In order to identify the fungus, we isolated hyphae from the plantlets for cultivation on potato dextrose (26.5 g/l potato dextrose bouillon, 1% phyto agar, pH 5.8) and malt extract agar (3% malt extract, 1% phyto agar, pH 5.8) in an incubator in the dark at 28°C (Fig.1).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from mycelium grown in potato dextrose broth following a modified protocol by Möller et al (1992) and precipitated with isopropanol. The mixture was left overnight at –20°C, spun down, and the DNA pellet washed three times with 70% ethanol to ensure salt-free DNA. The DNA was amplified using Phusion® polymerase according to the LifeTechnologies instruction manual. The primers (0.5 µM) used for both PCR and sequencing were ITS1F & ITS4 and NS3 & NS8 (White et al. 1990), LROR & LR7 (Vilgalys & Hester 1990), and fRPB2-5F, -7cR, -7cF, & -11aR (Liu et al. 1999). PCR conditions were initial denaturation at 98°C for 30 s, 35 cycles of 98°C for 10 s, appropriate annealing temperature for 30 s, and 72°C for 1 min followed by a final elongation at 72°C for 5 min. Amplification was carried out in an Eppendorf Mastercycler ep gradient S. PCR products were separated on a 1xTAE gel, cut out, extracted using a GE Healthcare Gel Extraction Kit, and sequenced at Eurofins MWG (Ebersberg, Germany).

Total DNA was isolated from plants as described above and checked with the appropriate fungal primers under the same PCR conditions except for an annealing temperature of 68°C. The PCR fragments were also sequenced and compared with the known rDNA sequence of the endophytic fungus to confirm the presence of the fungus in the plants.

Phylogenetic analysis

Sequences of the nuclear internal transcribed spacer region (ITS, including ITS1+5.8S rDNA+ITS2), the large (28S) and small (18S) nuclear rDNA subunits, and the second largest RNA polymerase II (RPB2) subunit from *Cyanodermella asteris* were run in a BLASTn search (Altschul et al. 1990). The different DNA regions were also used to build a phylogeny including closely related fungi. For this, the ITS, 18S, 28S and RPB2 sequences from *C. asteris* and other fungi representing the *Stictidaceae* (Table 1) were aligned using the Guidance2 Server (Landan & Graur 2008, Penn et al. 2010, Sela et al. 2015) with the default settings (including MAFFT algorithm) and *Phlyctidaceae* and *Coenogoniaceae* species as out-group. After sequence alignment, phylogenetic trees were generated from single region and combined sequences using MEGA6.0 (Tamura et al. 2013). The phylogenetic trees were inferred using the Maximum Likelihood method based on the General Time Reversible (Nei & Kumar 2000) or the Kimura-2-parameter model (Kimura 1980). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The bootstrap method was used with 1000 replicates (Felsenstein 1985).

The combined alignment and phylogenetic tree do not include all species, because rDNA cluster sequences were incomplete for some (Table 1).

Microscopy

The fungus was examined under an AP-8 binocular microscope (Thalheim Spezial Optik, Pulsnitz, Germany) and tissue samples were inspected with transmitted light with an Axiolab microscope (Zeiss, Jena, Germany). Additionally, fungal hyphae from a shake culture were stained with 1 g Congo Red in 100 ml 5% SDS to visualize fungal cell walls and septa (Clémençon 2009) under the microscope.

Growth tests

Malt extract broth (50 ml) was inoculated with 0.5 ml of homogenized hyphae of *Cyanodermella asteris* and incubated at 180 rpm. The medium was buffered in the pH test with 50 mM phosphate or 50 mM citrate buffer and cultures were incubated at 22°C for 10 d. The cultures of the temperature test were incubated in unbuffered medium (pH 5.3) for 17 d. Growth rates (mm/day) were recorded from 42 d old cultures on potato dextrose and malt extract agar plates kept at 23°C in the dark. Colors were coded according to Munsell (1905).

Table 1. DNA sequences from *Stictidaceae* used for phylogenetic trees

Species	* = isolate ^ = voucher	18S	ITS	28S	RPB2
Absconditella lignicola	*EB211	—	FJ904669.1	FJ904669.1	—
	Svensson & Baloch SW187 (S)	—	—	—	HM244776.1
A. rubra	^van den Boom 52517	KT454800.1	KT454800.1	—	—
A. sp.	*Spribille 39168	KR017250.1	KR017125.1	KR017188.1	KR017501.1
	^GS5_2_17	—	KF128882.1	—	—
	^Palice 3820	—	—	AY300825.1	—
A. sphagnorum	*M24	EU940022.1	JX298897.1	EU940095.1	EU940311.1
Acarosporina microspora	*AFTOL 78	AY584667.1	DQ782834.1	AY584643.1	AY584682.1
Bryodiscus arctoalpinus	^Baloch SW057	—	—	HM244760.1	HM244781.1
Carestiella socia	*GG2410	—	AY661687.1	AY661687.1	—
	^Wedin 7194 (UPS)	—	—	—	HM244782.1
Coenogonium leprieurii	^Kauff pa04021998-522	AF465457.1	—	AF465442.1	AY641032.1
C. luteum	^Ryan 31430 (ASU)	AF279386.1	—	AF279387.1	AY641038.1
	*AFTOL 352	—	HQ650710.1	—	—
C. pineti	^HB Palice	—	—	AY300834.1	—
	^Thor 19164 (UPS)	—	—	—	HM244786.1
Conotrema populorum	^UME41471	U86582.1	—	—	—
	*GG2610a	—	AY527327.1	—	—
	^Gilenstam 2353 (UPS)	—	—	AY300833.1	—
C. sp.	*MW7200		AY527313.1	AY527313.1	—
	*AN3222	—	AY527336.1	AY527336.1	—
Cryptodiscus foveolaris	*EB155	—	FJ904673.1	—	—
	*GG2603a	—	—	AY661683.1	—
	^Baloch & Arup SW166 (S)	—	—	—	HM244787.1
C. gloeocapsa	*EB93	FJ904696.1	FJ904674.1	—	—
	^Tibell 23543 (UPS)	—	—	—	HM244788.1
C. incolor	*S:F116574	—	NR_121357	—	—
C. pallidus	*EB152	—	FJ904679.1	FJ904679.1	—
	^Baloch & Wedin SW174 (S)	—	—	—	HM244789.1
C. pini	^Baloch & Arup SW175 (S)	—	HM244762.1	HM244762.1	HM244790.1
C. rhopaloides	*EB100	—	FJ904685.1	—	HM244791.1
*C. sp.*uncultured	clone F9	—	KP323396.1	—	—
Cyanodermella asteris	**strain 03HOR06-2-4**	**KT758843**	**KT758843**	**KT758843**	**KU934214**

Species	* = isolate ^ = voucher	18S	ITS	28S	RPB2
C. oleoligni	strain DTO 301-G1	KX999145.1	KX950434.1	KX950461.1	KX999147.1
C. viridula	^E. & C. Baloch SW129 (S)	—	—	HM244763.1	HM244792.1
	^UME29146	U86583.1	—	—	—
Glomerobolus gelineus	*AFTOL 1349	—	DQ247782.1	—	DQ247793.1
	strain JK 5548K	DQ247811.1	—	—	—
	strain JK 5584C	—	—	DQ247798.1	—
Odontotrema phacidiellum	^Gilenstam 2625 (UPS)	—	—	HM244769.1	HM244802.1
O. phacidioides	^Palice 11440 (S)	—	—	HM244770.1	HM244803.1
Ostropa barbara	*AFTOL 77	AY584666.1	—	AY584642.1	—
	^Wedin & Baloch SW071 (S)	—	HM244773.1	—	HM244806.1
Petractis luetkemuelleri	^Nimis & Tretiach 2000 (TSB 31659)	AF465461.1	—	AF465454.1	—
	*AFTOL 381	—	HQ650714.1	—	—
	^Geletti & Tretiach 1995 (TBS)	—	—	—	AY641061.1
P. nodispora	^A.Orange 17559 (NMW [C.2007.001.282])	FJ588712.1	—	FJ588713.1	—
	*NMW C.2007.001.284	—	NR_120312.1	—	—
Phlyctis agelaea		—	—	HQ659177.1	—
	^PHLAGE08257	—	—	—	KC020280.1
P. argena	*AFTOL 1375	DQ986725.1	—	DQ986771.1	DQ992458.1
	*BP8	—	KJ409433.1	—	—
Schizoxylon albescens	^ Gilenstam 2696a (UPS)	DQ401142.1	NR_121319.1	DQ401144.1	—
	^Wedin 7919 (UPS)	—	—	—	HM244813.1
S. sp.	*GG2365	—	AY661689.1	AY661689.1	—
Stictis brunnescens	*GG2359	—	AY661688.1	AY661688.1	—
	^Wedin 7651 (UPS)	—	—	—	HM244814.1
S. confusa	^Wedin 7070 (UPS)	—	DQ401143.1	DQ401143.1	HM244815.1
S. populorum	^UME41471	U86582.1	—	—	—
	*MW7301	—	AY527334.1	—	—
	^Gilenstam 2353 (UPS)	—	—	AY300833.1	—
	^Wedin 7626 (UPS)	—	—	—	HM244817.1
S. radiata	^Palice (ESS 21520)	AY300864.1	—	—	—
	*AFTOL 398	—	DQ782846.1	—	—
	^Jamie Platt JP222	—	—	AF356663.1	AY641079.1
S. sp.	*GG2445a	—	AY527318.1	AY527318.1	—
	*GG2440b	—	AY527321.1	—	—
	*GG2620b	—	AY527332.1	AY527332.1	—
S. urceolatum	*AFTOL 96	DQ983488.1	HQ650601.1	—	DQ992478.1

Taxonomy

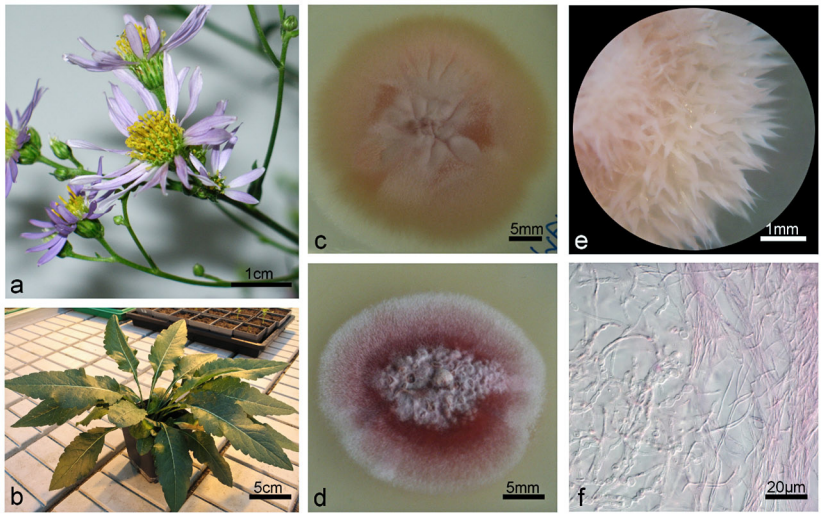

Fig. 2. The endophytic fungus *Cyanodermella asteris*. a, b. Habitus of the host *Aster tataricus* showing inflorescences and ground rosette; c. 55 d-old growth on malt extract agar; d. 55 d-old growth on potato dextrose agar; e. filamentous structure of the colony (36 d old); f. hyphae stained with Congo Red.

Cyanodermella asteris L. Jahn & Ludw.-Müll. **sp. nov.** Fig. 2

MycoBank MB 814158

Differs from *C. viridula* by its endophytic habit and its rose-pink hyphae in culture.

Type—Germany, Saxony, Dresden, isolated as endophyte of *Aster tataricus* cv. Austria, 21 Mar 2013, L. Jahn, **holotype** Herb. DR 043292 (metabolically inactive), living culture at DSMZ under DSM 100826.

Etymology—The epithet 'asteris' is derived from the host plant, *Aster tataricus* cv. Austria.

Sexual stage unknown. Colonies (14 d old) on potato dextrose agar white (N 9) to pale pink (2.5R 9/2); in age fading from pale pink to light pink (2.5R 8/6); after 30 days, becoming white towards the margin and turning light and deep pink (2.5R 6/10) to vivid red (5.0R 4/14) towards the center. Colors less intense on malt extract agar: 7–14 d old colonies usually colorless (N 9) and hardly distinguishable from the medium, >50 d old colonies colorless at the margin and light pink at the center.

Growth rate 0.7 ± 0.3 mm/day on potato dextrose agar and 0.9 ± 0.1mm/day on malt extract agar at an optimum temperature of 22–24°C and a pH of 5.8–6.

Host & distribution—The distribution of the endophytic fungus *C. asteris* may be linked to the distribution of its host, *A. tataricus,* which is native to northern Asia. *Cyanodermella asteris* has been isolated and detected by PCR in several plants of *A. tataricus* cv. Austria and also detected by PCR in *A. tataricus* plants obtained from the Botanical Garden Dresden, Germany, but which originated in Siberia. Isolation of *C. asteris* from these plants is currently in progress.

Phylogenetic results

The nuclear rDNA sequences of *Cyanodermella asteris* used for identification (as the "rDNA cluster") comprised the 18S (1539 bp), ITS (ca. 535 bp) and 28S (1359 bp) (GenBank KT758843). The first BLASTn search showed a distant relationship to the *Stictidaceae* lineage of the ostropalean fungi (Table 2). This led to a second BLASTn search against the *Stictidaceae* (Table 3). The 18S *C. asteris* sequence showed as nearest neighbor to *C. oleoligni* (98% identity) and *C. viridula* (95% identity), *Acarosporina microspora* (94% identity), and less closely related *Stictis* species. Similarly, the 28S *C. asteris* sequence revealed a relationship with *C. oleoligni* (94% identity) and *C. viridula* (95% identity). The ITS region showed a closest match to *C. oleoligni* (91% identity), *Stictis* sp. (96% identity), and *Carestiella socia* (96% identity); no ITS sequences were available for *C. viridula*.

Table 2. BLASTn results of the *C. asteris* rDNA cluster region

	C. asteris			BLASTn hits			
Loci	# BPs	GC %	Species	accession #	query cover	ident	E value
18S	1521	48.0	*Cyanodermella oleoligni*	KX999145.1	74%	98%	0.0
			Trapelia involuta	AF119499.2	78%	91%	0.0
			Trapelia placodioides	AF119500.2	78%	95%	0.0
			Acarospora smaragdula	AY552543.1	78%	91%	0.0
			Cyanodermella viridula	U86583.1	78%	91%	0.0
ITS	899	53.9	*Pleopsidium chlorophanum*	DQ525472.1	47%	84%	$4*10^{-97}$
			Acarospora insignis	LN890273.1	39%	87%	$6*10^{-95}$
			Acarospora smaragdula	EU870652.1	38%	86%	$4*10^{-97}$
28S	1329	49.4	*Cyanodermella oleoligni*	KX950461.1	89%	94%	0.0
			Umbilicaria crustulosa	HM161593.1	100%	90%	0.0
			Umbilicaria haplocarpa	HM161534.1	99%	90%	0.0

Table 3. BLASTn results of the *C. asteris* rDNA cluster region against *Stictidaceae*

loci	species	BLASTn hits			
		accession #	query cover	ident	E value
18S	*Cyanodermella oleoligni*	KX999145.1	74%	98%	0.0
	Cyanodermella viridula	U86583.1	77%	95%	0.0
	Acarosporina microspora	AY584667.1	76%	94%	0.0
	Stictis urceolatum	DQ983488.1	76%	94%	0.0
ITS	*Cyanodermella oleoligni*	KX950434.1	32%	91%	$2*10^{-95}$
	Stictis radiata	AY527308.1	30%	88%	$3*10^{-88}$
	Stictis brunnescens	AY661688.1	20%	96%	$3*10^{-84}$
	Cryptodiscus pini	FJ904682.1	28%	88%	$4*10^{-82}$
	Carestiella socia	AY661682.1	20%	96%	$4*10^{-82}$
28S	*Cyanodermella oleoligni*	KX950461.1	89%	94%	0.0
	Xyloschistes platytropa	KJ766680.1	97%	88%	0.0
	Stictis radiata	AY340575.1	77%	90%	0.0
	Acarosporina microspore	AY584643.1	83%	90%	0.0
	Cyanodermella viridula	HM244763.1	41%	95%	0.0

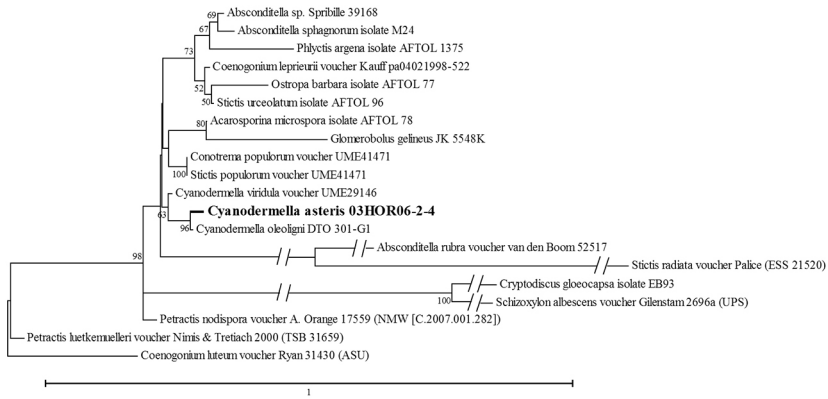
Absconditella sp. Spribille 39168
Absconditella sphagnorum isolate M24
Phlyctis argena isolate AFTOL 1375
Coenogonium leprieurii voucher Kauff pa04021998-522
Ostropa barbara isolate AFTOL 77
Stictis urceolatum isolate AFTOL 96
Acarosporina microspora isolate AFTOL 78
Glomerobolus gelineus JK 5548K
Conotrema populorum voucher UME41471
Stictis populorum voucher UME41471
Cyanodermella viridula voucher UME29146
Cyanodermella asteris 03HOR06-2-4
Cyanodermella oleoligni DTO 301-G1
Absconditella rubra voucher van den Boom 52517
Stictis radiata voucher Palice (ESS 21520)
Cryptodiscus gloeocapsa isolate EB93
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Petractis nodispora voucher A. Orange 17559 (NMW [C.2007.001.282])
Petractis luetkemuelleri voucher Nimis & Tretiach 2000 (TSB 31659)
Coenogonium luteum voucher Ryan 31430 (ASU)
1

Fig. 3. 18S sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the Kimura-2-parameter model (Kimura 1980). The tree with the highest log likelihood (–15822.6420) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.6859)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as out-group.

The RPB2 sequence (2059 bp, GenBank KU934214) was used to refine phylogenetic relationships within the *Stictidaceae*. Phylogenetic analyses based on the individual 18S, ITS, 28S, and RPB2 sequences of *Cyanodermella asteris* with other *Stictidaceae* strongly support a sister-relationship among *C. asteris, C. viridula,* and *C. oleoligni* (Figs 3–6). The combined ML tree also

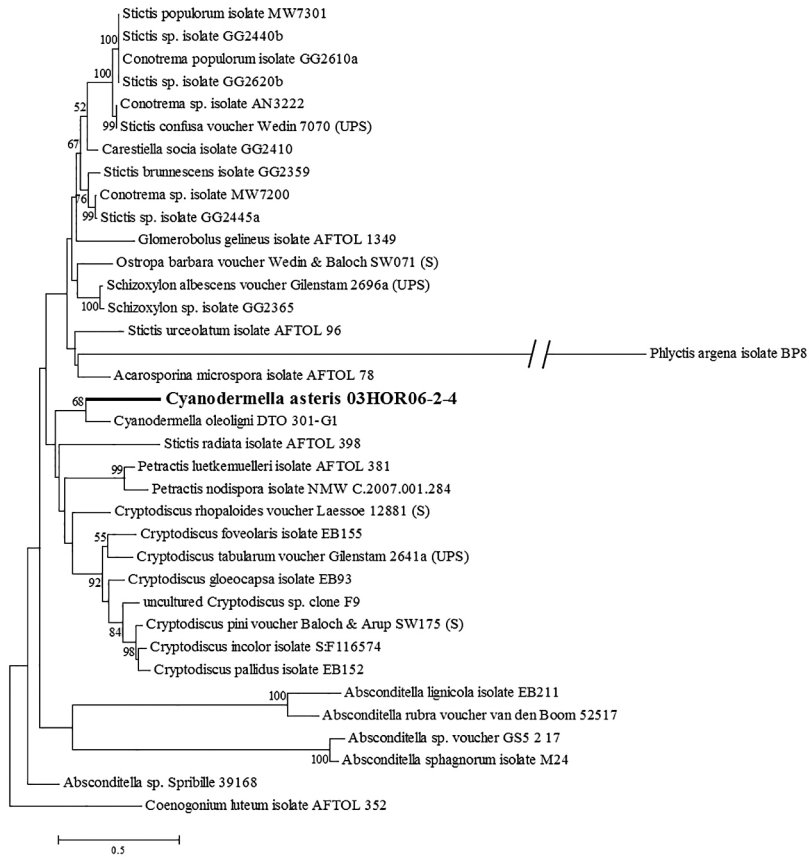
Stictis populorum isolate MW7301
Stictis sp. isolate GG2440b
Conotrema populorum isolate GG2610a
Stictis sp. isolate GG2620b
Conotrema sp. isolate AN3222
Stictis confusa voucher Wedin 7070 (UPS)
Carestiella socia isolate GG2410
Stictis brunnescens isolate GG2359
Conotrema sp. isolate MW7200
Stictis sp. isolate GG2445a
Glomerobolus gelineus isolate AFTOL 1349
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Schizoxylon sp. isolate GG2365
Stictis urceolatum isolate AFTOL 96
Phlyctis argena isolate BP8
Acarosporina microspora isolate AFTOL 78
Cyanodermella asteris 03HOR06-2-4
Cyanodermella oleoligni DTO 301-G1
Stictis radiata isolate AFTOL 398
Petractis luetkemuelleri isolate AFTOL 381
Petractis nodispora isolate NMW C.2007.001.284
Cryptodiscus rhopaloides voucher Laessoe 12881 (S)
Cryptodiscus foveolaris isolate EB155
Cryptodiscus tabularum voucher Gilenstam 2641a (UPS)
Cryptodiscus gloeocapsa isolate EB93
uncultured Cryptodiscus sp. clone F9
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Cryptodiscus incolor isolate S:F116574
Cryptodiscus pallidus isolate EB152
Absconditella lignicola isolate EB211
Absconditella rubra voucher van den Boom 52517
Absconditella sp. voucher GS5 2 17
Absconditella sphagnorum isolate M24
Absconditella sp. Spribille 39168
Coenogonium luteum isolate AFTOL 352
0.5

Fig. 4. ITS sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–11371.6910) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 1.4100)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* were used as out-group.

clusters *C. asteris* with the other two *Cyanodermella* species, but the combined phylogenetic tree is not as well supported as the others due to missing sequences (Fig. 7; Treebase TB2:S18310).

Discussion

Sequence analyses of the nuclear rDNA cluster from *Cyanodermella asteris* implied a close relationship with the *Stictidaceae* lineage of the *Ostropales* (*Lecanoromycetes*, *Pezizomycotina*, *Ascomycota*). The *Stictidaceae* are either saprotrophic or lichenized, and no stictidaceous species have been described as plant endophytes. However, Schulz & Boyle (2005) noted that

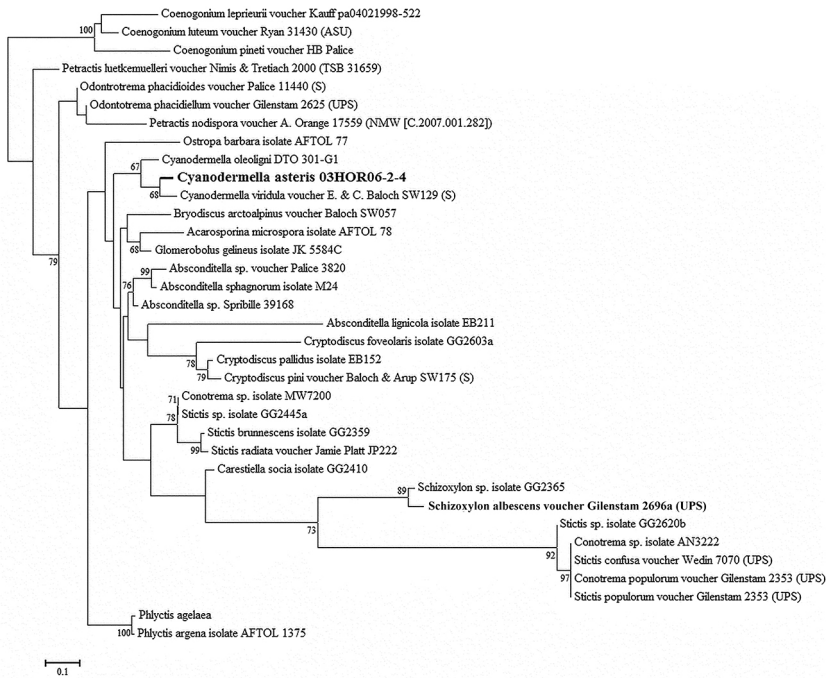
Coenogonium leprieurii voucher Kauff pa04021998-522
Coenogonium luteum voucher Ryan 31430 (ASU)
Coenogonium pineti voucher HB Palice
Petractis luetkemuelleri voucher Nimis & Tretiach 2000 (TSB 31659)
Odontotrema phacidioides voucher Palice 11440 (S)
Odontotrema phacidiellum voucher Gilenstam 2625 (UPS)
Petractis nodispora voucher A. Orange 17559 (NMW [C.2007.001.282])
Ostropa barbara isolate AFTOL 77
Cyanodermella oleoligni DTO 301-G1
Cyanodermella asteris 03HOR06-2-4
Cyanodermella viridula voucher E. & C. Baloch SW129 (S)
Bryodiscus arctoalpinus voucher Baloch SW057
Acarosporina microspora isolate AFTOL 78
Glomerobolus gelineus isolate JK 5584C
Absconditella sp. voucher Palice 3820
Absconditella sphagnorum isolate M24
Absconditella sp. Spribille 39168
Absconditella lignicola isolate EB211
Cryptodiscus foveolaris isolate GG2603a
Cryptodiscus pallidus isolate EB152
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Conotrema sp. isolate MW7200
Stictis sp. isolate GG2445a
Stictis brunnescens isolate GG2359
Stictis radiata voucher Jamie Platt JP222
Carestiella socia isolate GG2410
Schizoxylon sp. isolate GG2365
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Stictis sp. isolate GG2620b
Conotrema sp. isolate AN3222
Stictis confusa voucher Wedin 7070 (UPS)
Conotrema populorum voucher Gilenstam 2353 (UPS)
Stictis populorum voucher Gilenstam 2353 (UPS)
Phlyctis agelaea
Phlyctis argena isolate AFTOL 1375
0.1

Fig. 5. 28S sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–15372.4003) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.6449)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as out-group.

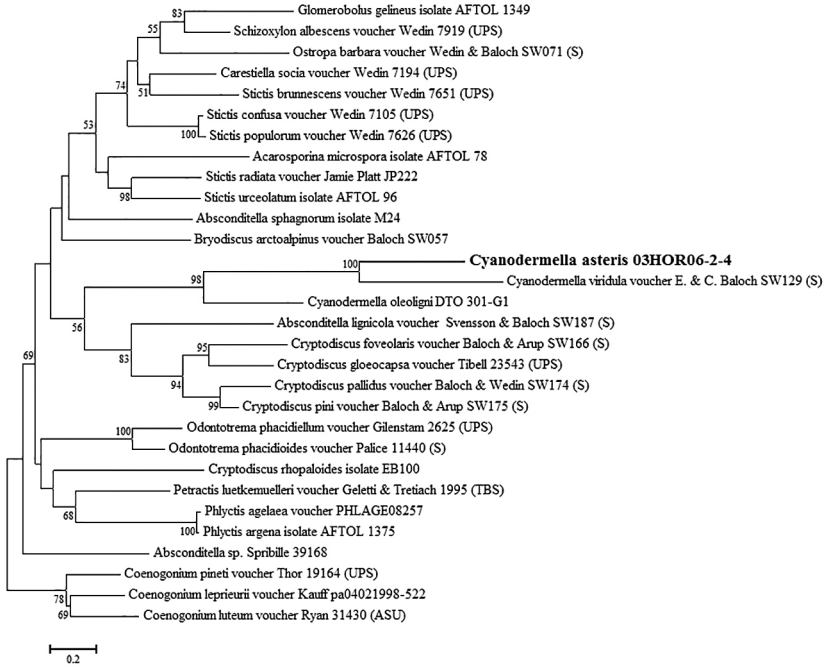
Glomerobolus gelineus isolate AFTOL 1349
Schizoxylon albescens voucher Wedin 7919 (UPS)
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Carestiella socia voucher Wedin 7194 (UPS)
Stictis brunnescens voucher Wedin 7651 (UPS)
Stictis confusa voucher Wedin 7105 (UPS)
Stictis populorum voucher Wedin 7626 (UPS)
Acarosporina microspora isolate AFTOL 78
Stictis radiata voucher Jamie Platt JP222
Stictis urceolatum isolate AFTOL 96
Absconditella sphagnorum isolate M24
Bryodiscus arctoalpinus voucher Baloch SW057
Cyanodermella asteris 03HOR06-2-4
Cyanodermella viridula voucher E. & C. Baloch SW129 (S)
Cyanodermella oleoligni DTO 301-G1
Absconditella lignicola voucher Svensson & Baloch SW187 (S)
Cryptodiscus foveolaris voucher Baloch & Arup SW166 (S)
Cryptodiscus gloeocapsa voucher Tibell 23543 (UPS)
Cryptodiscus pallidus voucher Baloch & Wedin SW174 (S)
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Odontotrema phacidiellum voucher Gilenstam 2625 (UPS)
Odontotrema phacidioides voucher Palice 11440 (S)
Cryptodiscus rhopaloides isolate EB100
Petractis luetkemuelleri voucher Geletti & Tretiach 1995 (TBS)
Phlyctis agelaea voucher PHLAGE08257
Phlyctis argena isolate AFTOL 1375
Absconditella sp. Spribille 39168
Coenogonium pineti voucher Thor 19164 (UPS)
Coenogonium leprieurii voucher Kauff pa04021998-522
Coenogonium luteum voucher Ryan 31430 (ASU)
0.2

Fig. 6. RPB2 sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–29390.8544) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5486)]. The percentage of trees clustering the associated taxa together is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as outgroup.

many endophytes are facultative in that they adopt a saprotrophic strategy as soon as their host plant dies. Although *C. asteris* did not visibly develop any fruit bodies or other structures on dying and dead plant material of *Aster tataricus,* a saprotrophic habit cannot be excluded.

The species within the *Stictidaceae* live in different ecological niches over the whole world (see Sherwood 1977a, b, Eriksson 1967, 1981, Wedin et al. 2006, Czarnota, Kukwa 2008, Baloch et al. 2009; Fig. 8). Nonetheless, no *Stictidaceae* have been recorded from Siberia or Mongolia. Our species is

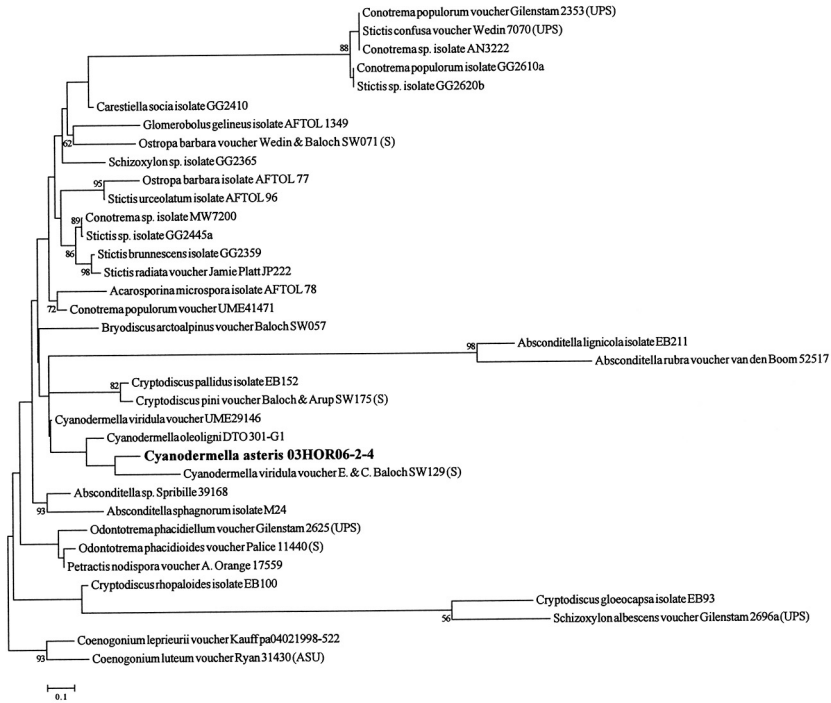
Conotrema populorum voucher Gilenstam 2353 (UPS)
Stictis confusa voucher Wedin 7070 (UPS)
88
Conotrema sp. isolate AN3222
Conotrema populorum isolate GG2610a
Stictis sp. isolate GG2620b
Carestiella socia isolate GG2410
Glomerobolus gelineus isolate AFTOL 1349
62
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Schizoxylon sp. isolate GG2365
95
Ostropa barbara isolate AFTOL 77
Stictis urceolatum isolate AFTOL 96
89
Conotrema sp. isolate MW7200
Stictis sp. isolate GG2445a
86
Stictis brunnescens isolate GG2359
98
Stictis radiata voucher Jamie Platt JP222
Acarosporina microspora isolate AFTOL 78
72
Conotrema populorum voucher UME41471
Bryodiscus arctoalpinus voucher Baloch SW057
98
Absconditella lignicola isolate EB211
Absconditella rubra voucher van den Boom 52517
82
Cryptodiscus pallidus isolate EB152
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Cyanodermella viridula voucher UME29146
Cyanodermella oleoligni DTO 301-G1
Cyanodermella asteris 03HOR06-2-4
Cyanodermella viridula voucher E. & C. Baloch SW129 (S)
Absconditella sp. Spribille 39168
93
Absconditella sphagnorum isolate M24
Odontotrema phacidiellum voucher Gilenstam 2625 (UPS)
Odontotrema phacidioides voucher Palice 11440 (S)
Petractis nodispora voucher A. Orange 17559
Cryptodiscus rhopaloides isolate EB100
Cryptodiscus gloeocapsa isolate EB93
56
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Coenogonium leprieurii voucher Kauff pa04021998-522
93
Coenogonium luteum voucher Ryan 31430 (ASU)
0.1

Fig. 7. Combined rDNA cluster + RPB2 sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–50824.7554) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.6150)]. The percentage of trees clustering the associated taxa together is shown next to the branches. *Coenogoniaceae* were used as out-group.

both the first to inhabit a plant with origins in northern Asia and the first isolated endophyte representing the *Stictidaceae*.

It should be noted that within the *Ostropales,* the *Stictidaceae* are poorly sampled with relatively few sequences available to generate a phylogeny. The nearest neighbors of *C. asteris* were identified as *C. viridula* (Berk. & M.A. Curtis) O.E. Erikss. and *C. oleoligni* van Nieuwenhuijzen & Samson. The only sequences available for *C. viridula*—the 18S and 28S—show a 94% identity match with those from *C. asteris.* The more diverse ITS region—usually used

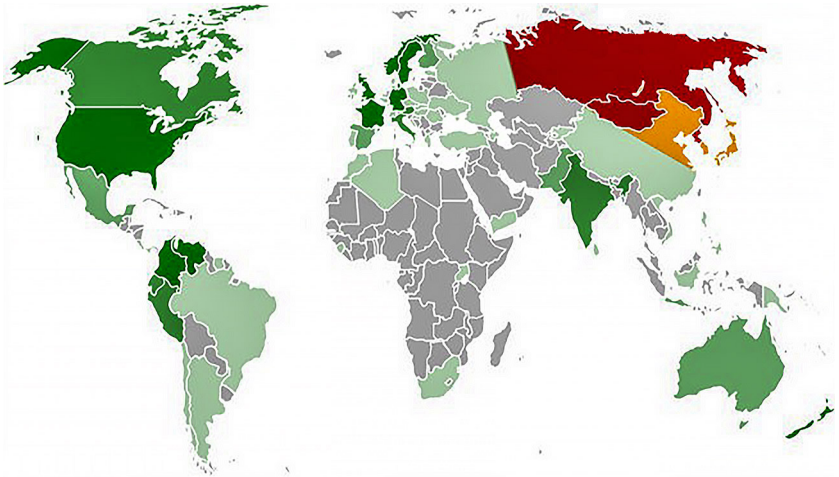

Fig. 8. Worldwide distribution of the *Stictidaceae* and *Aster tataricus* (Sherwood 1977a, b, Eriksson 1967, 1981, Wedin et al. 2006, Czarnota, Kukwa 2008, Baloch et al. 2009, Flann 2009). *Aster tataricus* is native to northern Asia including Siberia, Mongolia, Japan, Korea, and northern China, from which only a few *Stictidaceae* are described. Legend: Areas with >15 (●), 10–14 (●), 5–9 (●), or 1–4 (●) different species of *Stictidaceae;* native distribution of *Aster tataricus* (●); overlapping areas (●) and (●) are coded (●).

to identify and classify unknown fungal species (Schoch et al. 2012)—is not yet published from *C. viridula*. *Cyanodermella oleoligni* shows slightly higher similarities with *C. asteris* than *C. viridula*.

Cyanodermella is characterized by its perithecoid apothecia (Höhnel 1919; Eriksson 1967, 1981), which are very small and closed (Baloch et al. 2010). Only three species are described so far: *C. oleoligni, C. viridula*, and *C. candida* (Setch.) O.E. Erikss.

Cyanodermella viridula is known from old leaves of *Leymus arenarius* on beaches found in Sweden and western Russia near St. Petersburg as well as on twigs in North America, while *C. candida* is known only on ferns in Mexico (Eriksson 1967, 1981). *Cyanodermella oleoligni* (van Nieuwenhuijzen et al. 2016), which was isolated from oil-treated timber of *Pinus sylvestris,* grows slowly in grey to green colonies on fungal media. With the sexual stage of *C. asteris* not yet known despite an intensive search for ascomata on living and dead plant material, we expect its classification to be refined as more sequences from the *Stictidaceae* become available.

The role of *C. asteris* in or for the host plant is not yet clear. Because no endophyte-free cultivars of *A. tataricus* are available to us, we are unable to determine whether the fungus aids the plant's growth in the environment. Schulz & Boyle (2005) and Rodriguez et al. (2009) report that many endophytic fungi do not promote growth even when the plant is under stress. We speculate that if host plant serves as a habitat to protect *C. asteris* from the environment, the need for the fungus to adjust to the host plant might explain the initially slow growth (later somewhat accelerated; data not shown) of *C. asteris* in culture media. Future research will enable us to determine which metabolic and other features are characteristic for *C. asteris*.

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